**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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|---|---|---|---|
| Applicants: | Dharmaraj Ramachandra Rao, *et al.* | § | |
| | | § | Group Art Unit: 1626 |
| Serial No.: | 10/539,415 | § | |
| | | § | Examiner: Yong Liang Chu |
| Filed: | March 20, 2006 | § | |
| | | § | Confirmation No.: 2363 |
| For: | PROCESS FOR PREPARING DULOXETINE AND INTERMEDIATES FOR USE THEREIN | § | |

**DECLARATION UNDER 37 CFR § 1.132**

I, Dharmaraj Ramachandra Rao, hereby declare and say that:

1. I am a co-inventor of the invention claimed in the above-identified patent application.

2. Attached as Exhibit A is a report describing the preparation and characterization of compounds of duloxetine.

3. Exhibit A depicts the results of chiral HPLC chromatographic analysis of a racemic mixture of duloxetine maleate which demonstrates two peaks corresponding to the R and S isomers, Figure 1, and (+) duloxetine maleate prepared as described in the attached experimental report, Figure 2. Additionally, Applicants provide a chromatograph of the blank run of chiral HPLC to show that there is no interference from the chiral column or the mobile phase at the retention time of the two isomers, Figure 3.

4. Figure 2 of Exhibit A demonstrates (+) duloxetine maleate prepared in accordance with the experimental report when subjected to chiral HPLC demonstrates a single peak corresponding to the S-isomer of duloxetine maleate with a $[\alpha]_{589} = +98°$ at C=1 in methanol.

5. The prior art reference cited by the Examiner, Robertson et al., U.S. Patent No. 4,956,388 (hereinafter *Robertson*) in Example 14 discloses a (+) duloxetine maleate composition having a $[\alpha]_{589} = +82°$ at C=1 in methanol. On the basis of this value Applicants calculated the optical purity (Op) of the *Robertson* composition as follows: $O_p = [+82°]_{observed} / [+98°]_{max} \times 100\% = 84\%$. Thus, the compositions of *Robertson's* have an optical purity of 84% with respect to the (+) isomer.

6. I, Dharmaraj Ramachandra Rao, further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: 18th May 2009.

Dharmaraj Ramachandra Rao

**13th May 2009**

**Repeat of Example-3 of US appln. No. 10/539415 (Experiment No- CRD/451/1)**

(±) Duloxetine (10 gms) was dissolved in methanol and (-)di-p-tolyl tartaric acid (3.25 gms) was added. The reaction mass was refluxed under stirring for 1 hr. Methanol was distilled from the reaction mass under vacuum. Acetone (75 ml) was added to the residue and cooled to 5C to give the (-) di-p-toluyl tartrate salt of (+) duloxetine (6 gms). The tartrate salt was suspended in a mixture of water and toluene and sodium hydroxide was added. The organic layer was separated, dried and concentrated under vacuum to residue to give (+) duloxetine base.

This residue was stirred in ethyl acetate and the resulting solid was filtered and dried under vacuum at 50C to give 4.5 gm of (+) duloxetine base.

**Analysis:-**

Chiral HPLC Purity (100%)

**Preparation of (+)Duloxetine Maleate Experiment No- CRD/369/235**

(+) duloxetine 13 gms was stirred in 150 ml ethyl acetate at 25C, and heated to 45-50 C. Maleic acid (5.2 gms) was dissolved in 150 ml of ethyl acetate at 25C.
Maleic acid solution was added to the above heated solution at 50 C. The reaction mass was stirred at 50 C for 1 hr and later cooled to 25 C. The resulting solid was filtered and washed with 100 ml ethyl acetate and dried at 45 C under vacuum for 15 hrs to give 10.5 gms of (+)duloxetine maleate.

**Analysis:-**

Chiral HPLC Purity (100 %) $[\ ]_{589}$ = +98 at c=1 methanol as shown in Figure 2.

**Optical purity and enantiomeric excess calculation.**

The classical old method for calculating optical purity of a compound from the value of rotation is used for academic interest. At present more reliable techniques like Chiral HPLC are employed to check chiral purity, nevertheless optical purity of a chiral compound is expressed as the percentage ratio of the rotation observed and the maximum rotation (rotation of a pure enantiomer).

We have synthesized (+) duloxetine maleate in lab and checked the chiral HPLC which shows the single peak (see Figure 2) corresponding to the (+) isomer ($[\ ]_{589}$ = +98 at c=1 MeOH), also we have compared it with the chiral HPLC of racemic mixture of duloxetine which shows two peaks of (+) and (-) isomer (see Figure 1).

From the chiral HPLC it is clear that (+) duloxetine maleate prepared is substantially free of (-) isomer and can be considered as a pure enantiomer (see Figure 2). Other factors which can affect the rotation value were also taken into account like the moisture content, assay of the compound, HPLC purity, residue on ignition & residual solvent. All these parameters were analysed and accounted for when the rotation of (+) duloxetine maleate was done.

Optical purity can be calculated by the formula as given below

$$O_p = [\ ]_{observed} / [\ ]_{max} \times 100$$

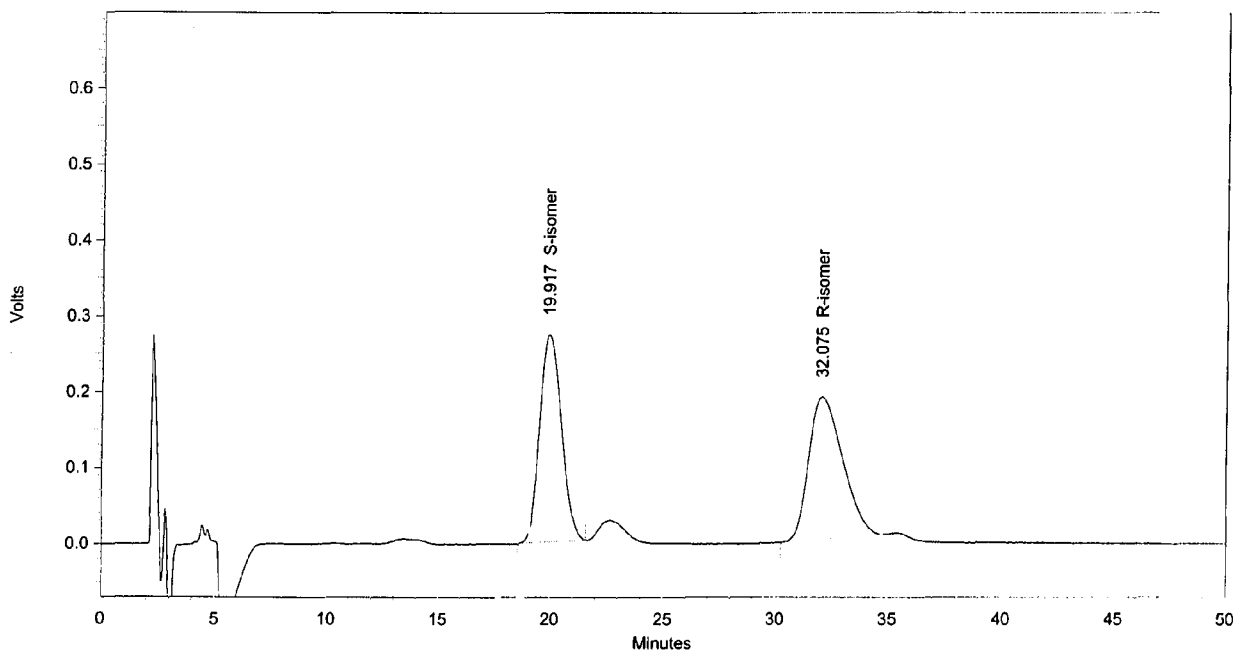
In Example 14 of US4956388 rotation of (+) duloxetine maleate is given as +82 then we can calculate the optical purity as follows

$$O_p = [\ +82\ ]_{observed} / [+98\ ]_{max} \times 100$$

$$= 84\ \%$$

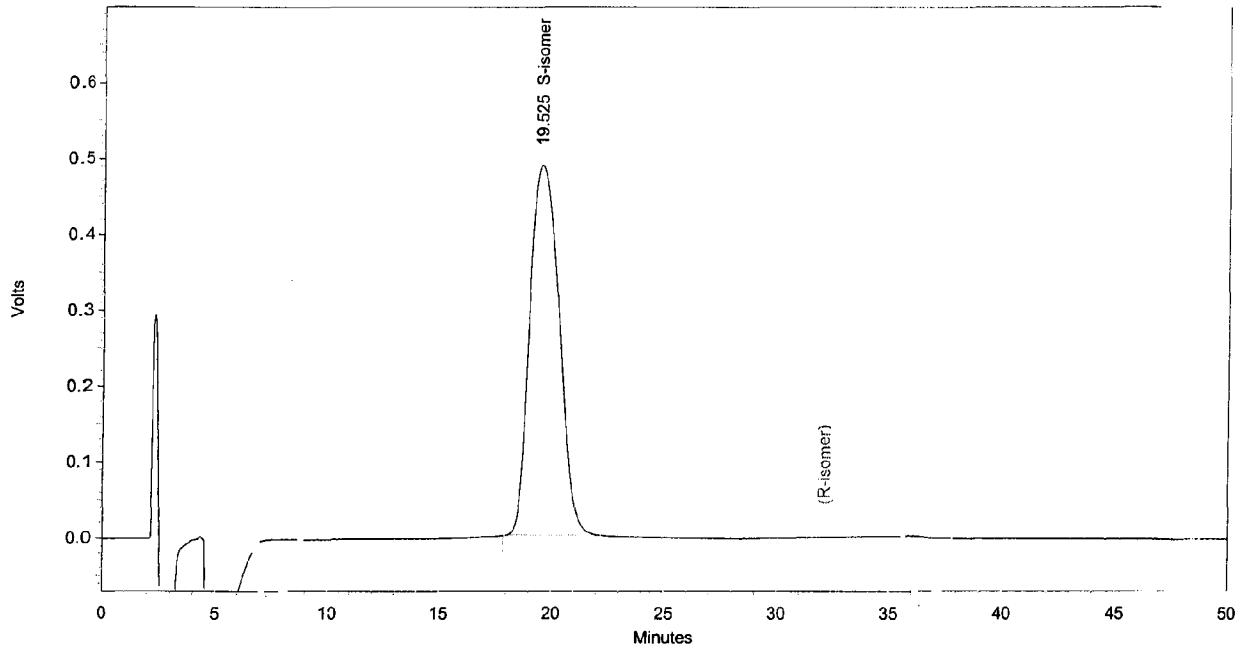
Thus it can be easily concluded that the compound prepared in example 14 is not enantiomerically pure and has an optical purity of 84 %.

Also we have repeated the Example 3 of our patent application and isolated (+) duloxetine base and checked its chiral HPLC purity and found to be 100 % and it is free of the (-) isomer and seen in the chromatogram.




| Detector A - 1 (220nm) Pk # | Retention Time | Area | Area % | Name | Resolution |
|---|---|---|---|---|---|
| 1 | 19.917 | 18228916 | 48.31 | S-isomer | 0.00 |
| 2 | 32.075 | 19500678 | 51.69 | R-isomer | 5.22 |
| Totals | | 37729594 | 100.00 | | |

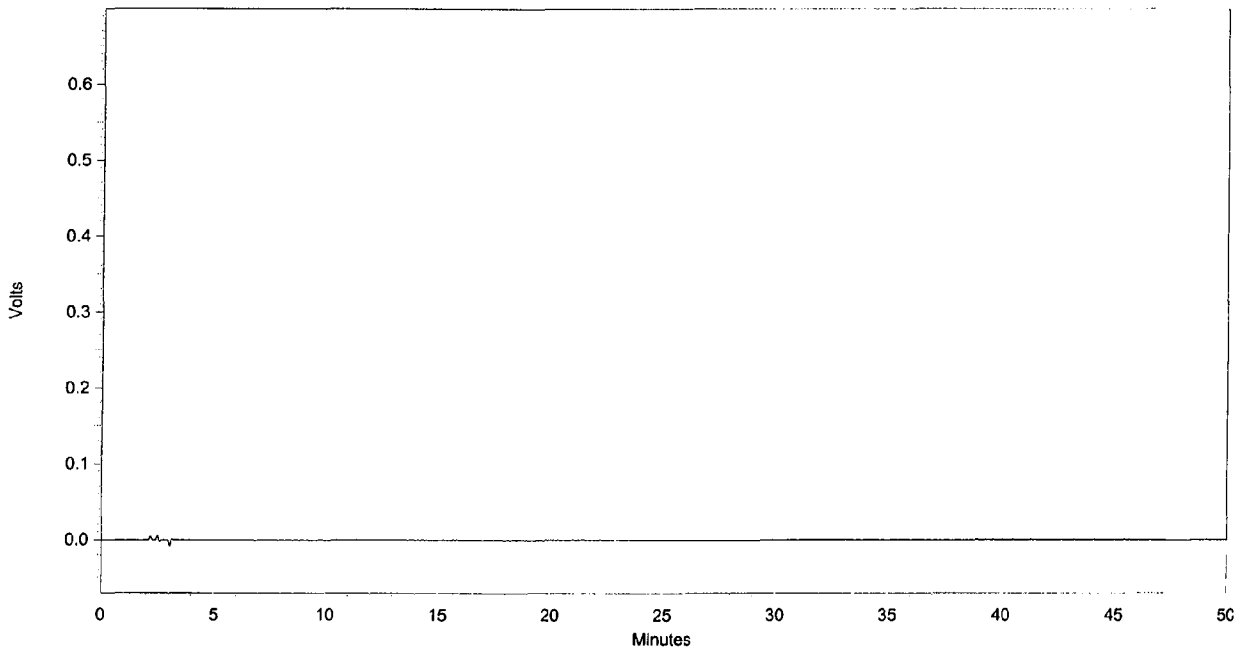
*FIG. 1*




**Detector A - 1 (220nm)**

| Pk # | Retention Time | Area | Area % | Name |
|---|---|---|---|---|
| 1 | 19.525 | 44383118 | 100.00 | S-isomer<br>R-isomer |
| Totals | | 44383118 | 100.00 | |

*FIG. 2*




| Detector A - 1 (220nm) Pk # | Retention Time | Area | Area % | Name | Resolution |
|---|---|---|---|---|---|

*FIG. 3*

**Cipla** **TECHNICAL INFORMATION SHEET**

**Vikhroli** **(Continuation Sheet)**

Item: Duloxetine maleate

Batch No. CRD-369-235

A. R. No. : VK09R2284

## SPECIFIC OPTICAL ROTATION

SAMPLE PREPARATION: 0.25075 gm of Sample is weighed and transferred in 25 ml volumetric flask Dissolved and diluted to the volume with Methanol.

Avg. SOR = + 97.507°

LOD ( By TGA) = 0.43%. w/w

## CALCULATION:

$$SOR = \frac{\text{Avg. SOR}}{(100 - \text{LOD})} \times 100$$

$$= \frac{97.507}{99.57} \times 100$$

$$= +97.93^{\circ}$$

$$= +98^{\circ}$$

Done by: Ganesh 07.05.2009

Date:

Checked by: Sanjay

Date: 12.05.09